Genetic Features of Natural and Cultured Populations in Plaice (Paralichthys olivaceus)

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Genetic Features of Natural and Cultured Populations in Plaice (*Paralichthys olivaceus*)

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Summary

To characterize genetically both natural population and cultured-released population of the plaice (*Paralichthys olivaceus*), enzyme polymorphism was examined by starch gel electrophoresis. Out of the examined 19 loci, 12 loci showed genetic variations. The screening of genetic variations at the 12 loci revealed no differences in the amount of genetic variability between natural and cultured-released populations in total. However, the quantification of genetic differentiation (Fst) revealed that the cultured-released population was significantly more diversified than the natural population, expecially, at the loci showing rare alleles. The divergence of cultured-released population was assumed to be promoted by the founder and/or bottleneck effect depending on the number of the parents kept in each hatchery.

Furthermore, it revealed the allelic and genotypic differences at the *Idh-1* showing polymorphism between natural and cultured-released populations. In natural population, an excess of homozygotes was observed in seven of 10 localities, suggesting that the natural population of plaice may be a mixed population. On the other hand, in cultured-released population, an excess of heterozygotes was observed in seven of the nine hatcheries, suggesting that the cultured-released population may have heterotic effect for survival rate.

Electrophoretic data has two major applications, one of them is the understanding the genetic population structure and the other examination of population mixture in fish resources. Several studies have dealt with the first of these two applications and confirmed the new insights on population structure of wild and cultured stocks in plaice (1), masu salmon (2), black rockfish (3) and abalone (4, 5).

The second application has relatively little attention for genetic monitoring of cultured-released population when hatchery fish were released into areas containing a natural population. Such cases have been reported in plaice (6) and black rockfish (3).

The plaice (*Paralichthys olivaceus*) distributed in coastal areas around Japan

is one of the important commercial fishes. This species has been cultured in several hatchery stations for release into natural environment. Such a situation in which a cultured-released population potentially contribute to a natural population leads the necessity to estimate how their relative contribution is. In the connection, Fujio *et al.* (1) demonstrated the genetic differences between natural and cultured-released populations of the plaice, indicating that the frequency of *A* allele at the liver isocitrate dehydrogenase (*Idh-1*) locus in natural population is 0.504 and in cultured-released population is 0.361. Such different frequencies are possible to estimate the proportion of individuals from each of the populations when they occur in a mixture. Fujio *et al.* (6) estimated the proportion of the released population to be 38.5% from the composition of the mixture when hatchery fish are released into a North-eastern area in Pacific Ocean side containing a wild population in 1986-1988.

The purposes of this work is to characterize genetically the natural and cultured-released populations of the plaice and discuss findings of the differences between natural and cultured-released population structures based on electrophoretic data.

Materials and Methods

Ten different samples from natural population of plaice (*Paralichthys olivaceus*) were collected from several locations along both Pacific Ocean and Japan Sea sides in Japan from 1983 to 1986. Nine different samples from the cultured-released population were provided from several hatchery stocks at the same birth in 1995. Samples were collected randomly from each stock for release. Standard body length and sampling data are shown in Table 1 and 2.

The isozymic markers for 12 enzymes were detected for the extracts of eye, muscle and liver by horizontal starch gel electrophoresis. The procedure of the electrophoresis and staining procedures were based on Fujio (7). The surveyed 12 enzymatic proteins were as follows; aspartate aminotransferase (AAT; E.C. No. 2.6.1.1), fructose-1, 6-diphosphatase (FDP : E.C. No. 3.1.3.11), α-glycerophosphate dehydrogenase (αGPD : E.C. No. 1.1.1.12), glucose-6-phosphate isomerase (GPI; E.C. No. 5.3.1.9), isocitrate dehydrogenase (IDH : E.C. No. 1.1.1. 42), lactate dehydrogenase (LDH : E.C. No. 1.1.1.27), malate dehydrogenase (MDH : E.C. No. 1.1.1.37), malic enzyme (ME : E.C. No. 1.1.1.40), mannose-6-phosphate isomerase (MPI : E.C. No. 5.3.1.8), 6-phosphogluconate dehydrogenase (6PGD : E.C. No. 1.1.1.44), phosphoglucomutase (PGM : E.C. No. 5.4.2.2), superoxide dismutase (SOD : E.C. No. 1.15.1.1). Alleles at each locus were designated by letters in alphabetical order, starting with the allele encording the most anodally migrating isozymes. Allele frequency data were used to elucidate the difference of the genetic variation between natural and cultured-released popula-

TABLE 1. *Collection Data of Natural Population of the Plaice in This Study*

Location	Sampling date	No of individuals	Standard Body Length Mean±SE (cm)
Pacific Ocean			
Aomori (Misawa)	1984.10.24	50	20.8±0.8
Iwate (Miyako)	1983. 9.30	50	21.6±1.5
Miyagi 1 (Sichigahama)	1983	50	19.0±3.7
Miyagi 2 (Watari)	1985. 6. 7	52	17.0±1.1
Fukushima (Haragama)	1986.10.10-30	145	23.3±2.4
Ibaragi	1984.12.19	48	13.5±1.1
Miyazaki	1986.10. 9	100	17.4±1.4
Japan Sea			
Hokkaido (Kobira)	198610.18	79	20.4±1.6
Tottori	1984.10. 1	46	23.4±1.1
Fukuoka (Hakata)	1986. 9.16-18	69	16.9±2.0

TABLE 2. *Collection Data of Cultured-released Population of the plaice in This Study*

Hatchery	Year of production	Sampling date	No. of individuals	Standard Body Length Mean±SD (cm)
Pacific Ocean				
Aomori	1995	1995. 9.11	110	5.7±0.5
Iwate	1995	1995.10.17	100	4.6±0.4
Miyagi	1995	1995.10.30	100	6.7±0.8
Fukushima	1995	1995.11. 2	100	9.2±0.5
Miyazaki	1995	1995.11.17	100	12.4±1.7
Japan Sea				
Akita	1995	1995. 4. 9	100	17.7±2.2
Kyoto	1995	1995.11.13	100	18.3±1.1
Tottori	1995	1995.11. 4	100	20.4±1.4
Kumamoto	1995	1995.11.15	40	22.7±1.6

tion in total. The fixation index (Fst) was also calculated from average and variance of allele frequencies in the natural and released-cultured populations for quantification of genetic differentiation among them. For the locus showing universal polymorphism which showed the frequency of the most common allele is no greater than 0.95 in all localities and hatcheries, the agreement between the observed and the expected numbers of genotypes under the Hardy-Weinberg's equilibrium were examined by χ^2-test in each locality or hatchery. An excess of either homozygotes or heterozygotes was expressed by d value which was calcu-

lated from the formura $d=(h_o-h_e)/h_e$, where h_o and h_e is observeved and expected heterozygosity. The positive and negative value of d indicates an excess of heterogyzotes and homozygotes, respectively.

Results

A total of 19 isozyme loci of plaice were detected in 19 different samples of natural and cultured-released populations of the plaice by starch-gel electrophoresis. Of the 19 loci examined, six loci (*Fdp*, *αGpd-2*, *αGpd-3*, *Me*, *Mpi* and *Sod*) were monomorphic in both natural and cultured-released populations, and *Ldh-3* loci were very rare variants; being observed in only three localities of natural populations. Allele frequencies at the remainng 12 loci which showed genetic variation in both of natural and cultured-released populations are shown in Table 3. Eleven loci (*Aat-1*, *Aat-2*, *αGpd-1*, *Gpi-1*, *Gpi-2*, *Idh-2*, *Ldh-2*, *Mdh-1*, *Mdh-2*, *6Pgd* and *Pgm*) showed rare variant alleles in at least one locality or one hatchery, and only one locus (*Idh-1*) showed polymorphism in over all localities and hatcheries in both of natural and cultured-released populations.

A comparison in the amount of genetic variability (H_e) was made between natural and cultured-released populations. Average of heterozygosity was ranged from 0.027 to 0.041 with a mean of 0.033 in natural population. On the other hand, average of heterozygosity ranged from 0.040 to 0.078 with a mean of 0.056 in cultured-released populations.

The result of the quantification of genetic differentiation (Fst) at the twelve loci between natural and cultured-released populations in total was shown in Table 4. A significant differences between natural and cultured-released populations were not observed in an average of allele frequencies, (t-test, $p>0.05$), except for the *6Pgd* locus. However, the Fst value of each allele was higher in the cultured-released population than in the natural population. Such phenomenon was significant at the loci showing rare alleles. Fst value means the degree of fluctuation among locality or hatcheries of natural and cultured-released populations. The locus shown rare variant, for example, the *Pgm* locus indicated high Fst value. While the *Idh-1* locus which showed polymorphism in all localities and hatcheries indicated low Fst value. The *D* allele frequency at the *Pgm* locus fluctuated from 0 to 0.007 with a mean of 0.001 in natural population. On the other hand, the *D* allele frequency fluctuated from 0 to 0.170 with a mean of 0.037 in cultured-released population (Fig. 1). In comparison with the *Pgm* locus, the *A* allele frequency at the *Idh-1* locus fluctuated from 0.333 to 0.561 with a mean of 0.470 in natural population. On the other hand, in cultured-released population, the *A* allele frequency fluctuated from 0.379 to 0.630 with a mean of 0.508 (Fig. 2).

The observed and expected number in each genotype at the *Idh-1* locus which

TABLE 3. *Allele Freguncies at 12 Loci in Natural and Cultured-released Population of the Plaice*

Locus	Allele	Natural population									
		Aomori (50)	Iwate (50)	Miyagi 1 (50)	Miyagi 2 (52)	Fukushima (145)	Ibaragi (50)	Miyazaki (100)	Hokkaido (79)	Tottori (46)	Fukuoka (69)
Aat-1	*A*	0.970	1.000	1.000	1.000	0.997	1.000	1.000	1.000	1.000	1.000
	B	0.030	0	0	0	0.003	0	0	0	0	0
Aat-2	*A*	0	0	0	0	0.003	0	0.010	0	0	0
	B	1.000	1.000	1.000	1.000	0.997	1.000	0.990	1.000	1.000	1.000
	C	0	0	0	0	0	0	0	0	0	0
aGpd-1	*A*	0.980	1.000	1.000	1.000	0.997	1.000	1.000	0.994	1.000	1.000
	B	0.020	0	0	0	0.003	0	0	0.006	0	0
Gpi-1	*A*	0	0.010	0	0	0.007	0	0.005	0	0	0.007
	B	1.000	0.990	1.000	1.000	0.990	0.950	0.985	0.987	0.967	0.986
	C	0	0	0	0	0.003	0.050	0.010	0.013	0.033	0.007
Gpi-2	*A*	0	0	0	0	0.003	0	0.010	0.013	0.011	0
	B	1.000	1.000	1.000	1.000	0.997	1.000	0.990	0.981	0.989	0.964
	C	0	0	0	0	0	0	0	0.006	0	0.036
Idh-1	*A*	0.480	0.489	0.558	0.510	0.469	0.531	0.439	0.333	0.543	0.413
	B	0.520	0.511	0.442	0.490	0.531	0.469	0.561	0.667	0.457	0.580
	C	0	0	0	0	0	0	0	0	0	0.007
Idh-2	*A*	0	0	0.011	0	0	0.010	0	0	0.015	0
	B	1.000	1.000	0.989	1.000	1.000	0.990	1.000	0.994	0.985	1.000
	C	0	0	0	0	0	0	0	0.006	0	0
Ldh-2	*A*	0.020	0	0	0	0	0	0	0.006	0	0
	B	0.980	1.000	1.000	0.963	0.997	0.990	0.985	0.988	1.000	0.978
	C	0	0	0	0.037	0.003	0.010	0.015	0.006	0	0.022
Mdh-1	*A*	0	0	0	0	0	0	0	0	0	0.007
	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.993
Mdh-2	*A*	0.010	0	0	0	0	0	0.005	0.006	0	0
	B	0.990	1.000	1.000	1.000	1.000	1.000	0.985	0.994	1.000	0.993
	C	0	0	0	0	0	0	0.010	0	0	0.007
6Pgd	*A*	0	0	0	0	0.003	0	0	0.006	0	0.007
	B	1.000	1.000	1.000	1.000	0.997	1.000	1.000	0.994	1.000	0.993
	C	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0
Pgm	*A*	0.010	0.050	0	0	0.007	0.020	0.005	0.006	0.022	0.007
	B	0	0	0	0	0	0	0	0	0	0
	C	0.990	0.950	1.000	1.000	0.990	0.980	0.995	0.994	0.978	0.986
	D	0	0	0	0	0.003	0	0	0	0	0.007
H_e		0.033	0.032	0.027	0.030	0.031	0.035	0.034	0.031	0.034	0.041

Sample size is in parenthesis under each loality of hetchery name. *Fdp*, *αGpd-2*, *αGpd-3*, *Me*, *Mpi* and *Sod* were monomorphic in over all populations. *Ldh-3* showed a rare variant allele in only three localities in natural population.

TABLE 3. *Continued.*

Locus	Allele	Cultured-released population								
		Aomori (110)	Iwate (100)	Miyagi (100)	Fukushima (100)	Miyazaki (100)	Akita (100)	Kyoto (100)	Tottori (100)	Kumamoto (40)
Aat-1	*A*	0.944	1.000	1.000	1.000	0.980	1.000	0.995	0.975	1.000
	B	0.056	0	0	0	0.020	0	0.005	0.025	0
Aat-2	*A*	0	0	0	0	0	0	0.030	0	0
	B	1.000	1.000	1.000	1.000	0.965	1.000	0.965	1.000	1.000
	C	0	0	0	0	0.035	0	0.005	0	0
aGpd-1	*A*	1.000	1.000	1.000	1.000	1.000	1.000	0.970	0.915	1.000
	B	0	0	0	0	0	0	0.030	0.085	0
Gpi-1	*A*	0.009	0	0	0	0	0	0.070	0	0
	B	0.982	0.995	1.000	1.000	1.000	1.000	0.930	1.000	1.000
	C	0.009	0.005	0	0	0	0	0	0	0
Gpi-2	*A*	0	0	0.005	0	0.080	0	0	0.025	0
	B	0.914	0.980	0.605	0.940	0.795	0.687	0.925	0.950	0.600
	C	0.086	0.020	0.390	0.060	0.125	0.313	0.075	0.025	0.400
Idh-1	*A*	0.630	0.457	0.420	0.540	0.410	0.530	0.505	0.600	0.379
	B	0.370	0.543	0.580	0.460	0.590	0.470	0.495	0.400	0.621
	C	0	0	0	0	0	0	0	0	0
Idh-2	*A*	0	0	0	0	0	0	0	0.060	0
	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.940	1.000
	C	0	0	0	0	0	0	0	0	0
Ldh-2	*A*	0.005	0	0	0	0	0	0	0	0
	B	0.995	1.000	1.000	0.890	1.000	1.000	1.000	1.000	1.000
	C	0	0	0	0.110	0	0	0	0	0
Mdh-1	*A*	0	0	0	0	0	0	0	0	0.025
	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.975
Mdh-2	*A*	0	0	0	0	0	0	0	0	0
	B	1.000	1.000	1.000	1.000	1.000	1.000	0.960	1.000	1.000
	C	0	0	0	0	0	0	0.040	0	0
6Pgd	*A*	0	0	0	0	0	0	0	0	0
	B	0.991	1.000	0.854	0.860	0.970	0.960	0.860	0.995	0.962
	C	0.009	0	0.146	0.140	0.030	0.040	0.120	0.005	0
	D	0	0	0	0	0	0	0.020	0	0.038
Pgm	*A*	0	0.026	0	0	0	0.010	0	0	0
	B	0	0	0	0.105	0	0	0	0	0
	C	1.000	0.881	0.960	0.725	1.000	0.970	1.000	1.000	0.987
	D	0	0.093	0.040	0.170	0	0.020	0	0	0.013
H_e		0.042	0.040	0.068	0.078	0.052	0.056	0.065	0.048	0.058

TABLE 4. *Mean Allele Frequencies at 12 Loci and Fst in Natural and Cultured-released Populations of Plaice*

Locus	Allele	Allele frequency (Mean±SD)		Fst	
		Natural	Cultured	Natural	Cultured
Aat-1	*A*	0.997±0.009	0.988±0.019		
	B	0.003±0.009	0.012±0.019	0.027	0.030
Aat-2	*A*	0.001±0.003	0.003±0.010	0.009	0.008
	B	0.999±0.003	0.993±0.015		0.032
	C	0	0.004±0.012		0.036
aGpd-1	*A*	0.997±0.006	0.987±0.029		
	B	0.003±0.006	0.013±0.029	0.012	0.054
Gpi-1	*A*	0.003±0.004	0.008±0.023	0.005	0.067
	B	0.986±0.016	0.990±0.023	0.019	0.053
	C	0.011±0.017	0.002±0.003	0.027	0.005
Gpi-2	*A*	0.004±0.005	0.012±0.027	0.006	0.061
	B	0.992±0.012	0.822±0.154	0.018	0.162
	C	0.004±0.011	0.166±0.156	0.030	0.175
Idh-1	*A*	0.477±0.068	0.497±0.087	0.019	0.030
	B	0.522±0.067	0.503±0.087	0.018	
	C	0.001±0.002	0	0.004	
Idh-2	*A*	0.004±0.007	0.007±0.020	0.012	0.058
	B	0.995±0.006	0.993±0.020	0.007	
	C	0.001±0.002	0	0.004	
Idh-2	*A*	0.003±0.006	0.001±0.002	0.012	0.004
	B	0.988±0.012	0.987±0.036	0.012	0.101
	C	0.009±0.012	0.012±0.037	0.016	0.115
Mdh-1	*A*	0.001±0.002	0.003±0.008	0.004	0.021
	B	0.999±0.002	0.997±0.008		
Mdh-2	*A*	0.002±0.004	0	0.008	
	B	0.996±0.005	0.996±0.013	0.006	
	C	0.002±0.004	0.004±0.013	0.008	0.042
6Pgd	*A*	0.002±0.003	0	0.005	
	B	0.998±0.003	0.939±0.062*		0.067
	C	0	0.055±0.062*		0.074
	D	0	0.006±0.014*		0.033
Pgm	*A*	0.013±0.015	0.004±0.009	0.018	0.020
	B	0	0.012±0.035		0.103
	C	0.986±0.015	0.947±0.092	0.016	0.169
	D	0.001±0.002	0.037±0.058	0.004	0.094

*Significant difference was oserved between the natural and cultured-released populations (t-test, $P<0.05$).

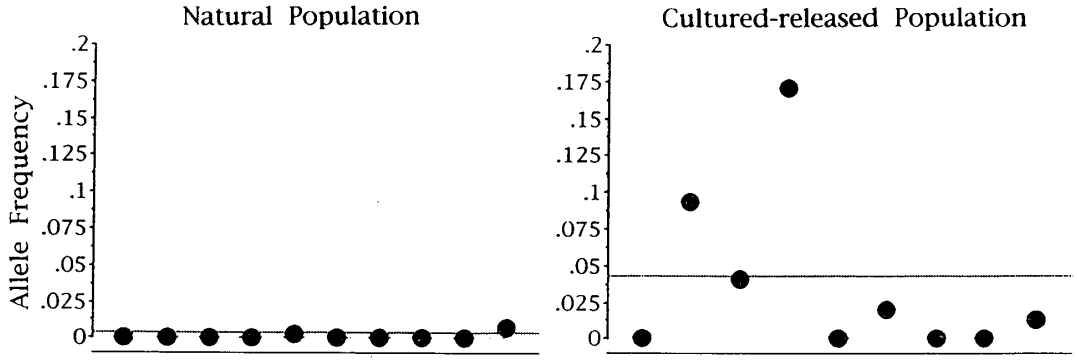

FIG. 1. The comparison of fluctuation of the Pgm^D allele between the natural and cultured-released populations of the plaice. A dotted line shows mean of allele frequency in each population.

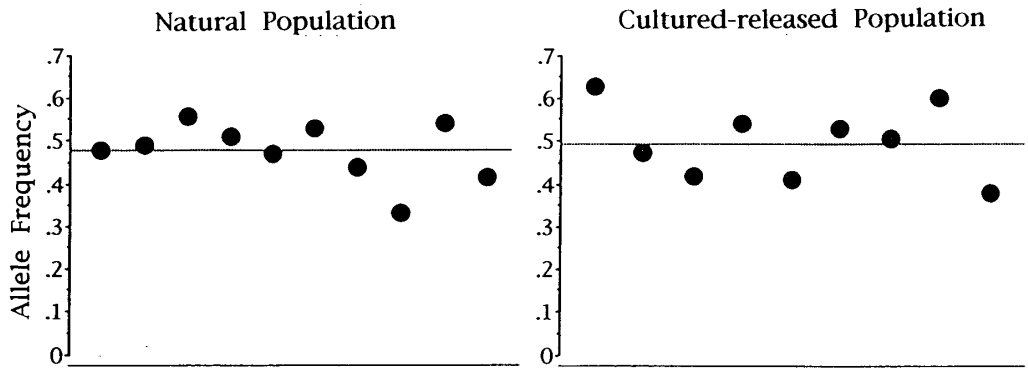

FIG. 2. The comparison in fluctuation of the $Idh\text{-}1^A$ allele between the natural and cultured-released populations of the plaice. A dotted line shows mean of allele frequency in each population.

showed polymorphism in both natural and cultured-released populations were shown in Table 5 and 6. An excess of homozygotes was observed in seven of 10 localities in natural population, while a excess of heterozygotes was observed in seven of the nine hatcheries in cultured-released population, and significant differences were observed in the three of those seven hatcheries.

Discussion

The present study demonstrated that the natural population of the plaice have maintained many rare alleles at a number of loci in each locality and a genetic differentiation have not occured within the species. A tendency of homozygotes excess at the *Idh-1* locus which showed polymorphism also suggests that the natural population of the plaice may be a mixed population. Kijima and Fujio (8) have reported that the quantity of genetic differentiation for the natural population of the plaice is lower than those obtained for natural popula-

TABLE 5. *Genotype and Allele Frequency at Idh-1 Locus in Natural Population of the Plaice*

Locality	Sample Size	Genotype Frequency				Allele Frequency			χ^2	d
		A/A	*A/B*	*B/B*	Others	q*A*	q*B*	q*C*		
Pacific Ocean										
Aomori	50	12(11.5)	24(25.0)	14(13.5)	0	0.480	0.520	0	0.080	−0.038
Iwate	45	13(10.8)	18(22.4)	14(11.8)	0	0.489	0.511	0	1.722	−0.200
Miyagi 1	26	6(8.1)	17(12.8)	3(5.1)	0	0.558	0.442	0	2.787	0.327
Miyagi 2	49	15(12.7)	20(24.5)	14(11.8)	0	0.510	0.490	0	1.653	−0.184
Fukushima	144	34(31.7)	67(71.6)	43(40.7)	0	0.469	0.531	0	0.816	−0.066
Ibaragi	48	14(13.5)	23(23.9)	11(10.6)	0	0.531	0.469	0	0.068	−0.038
Miyazaki	98	17(18.8)	52(48.3)	29(30.9)	0	0.439	0.561	0	0.573	0.072
Japan Sea										
Hokkaido	30	3(3.3)	14(13.3)	13(13.4)	0	0.333	0.667	0	0.760	0.052
Tottori	46	14(13.6)	22(22.8)	10(9.6)	0	0.543	0.457	0	0.057	−0.036
Fukuoka	69	13(11.8)	30(33.0)	25(23.2)	1(1.0)	0.413	0.580	0.007	0.534	−0.089
Total	605	141(133.6)	287(300.8)	176(169.3)	1(1.3)	0.470	0.529	0.001	1.308	−0.046

Expected number of genotype under the Hardy-Weinberg's equilibrium is indicated in parenthesis.
$d=(h_o-h_e)/h_e$

TABLE 6. *Genotype and Allele Frequency at Idh-1 Locus in Cultured-released Population of the Plaice*

Hatchery	Sample Size	Genotype Frequency			Allele Frequency		χ^2	d
		A/A	A/B	B/B	qA	qB		
Pacific Ocean								
Aomori	108	40(42.9)	56(50.3)	12(14.8)	0.630	0.370	1.372	0.114
Iwate	93	20(19.4)	45(46.2)	28(27.4)	0.457	0.543	0.063	−0.024
Miyagi	100	9(17.6)	66(48.8)	25(33.6)	0.420	0.580	12.465*	0.355
Fukushima	100	25(29.2)	58(49.6)	17(21.2)	0.540	0.460	2.859	0.167
Miyazaki	100	9(16.8)	64(48.4)	27(34.8)	0.410	0.590	10.397*	0.322
Japan Sea								
Akita	100	25(28.1)	56(49.8)	19(22.1)	0.530	0.470	1.549	0.124
Kyoto	100	18(25.5)	65(50.0)	17(24.5)	0.505	0.495	9.002*	0.300
Tottori	100	39(36.0)	42(48.0)	19(16.0)	0.600	0.400	1.470	−0.125
Kumamoto	33	3(4.7)	19(15.6)	11(12.7)	0.379	0.621	1.584	0.223
Total	834	188(215.2)	471(416.9)	175(201.9)	0.508	0.492	14.042*	0.130

Expected number of genotype under the Hardy-Weinberg's equilibrium is indicated in parenthesis.
*Significant difference ($P<0.05$); $d=(h_o-h_e)/h_e$.

tions of other Pleuronectid species. This species was characterized by a low level of genetic differentiation and a low level of average heterozygosity. The plaice have formed a large population with numerous breeding units of various effective sizes in many localities and their eggs and fly have been widely distributed and mixed well among localities.

On the other hand, the present study demonstrated that the quantity of genetic differentiation (Fst) for cultured-released population was higher than those obtained for natural population. This phenomenon is similar to that reported for natural and cultured populations of masu salmon (2), black rockfish (3), red seabream (9, 10), and Pacific herring (11). This suggests that varied sampling of parents can lead to a change in allele frequency compared that of natural population. Especially, production of the cultured-released population is dependent on the random selection of parents from the natural population, and each cultured-released population may be formed a sample of a few parents. Therefore, the divergence of the cultured-released population in the present study is assumed to be promoted by founder and/or bottleneck effects. This fact is important for the management of seed production, especially for natural population which is maintained as a genetic resources.

Furthermore, a tendency of heterozygotes excess at the *Idh-1* locus in

cultured-released population suggests that the selective advantage of heterozygotes may be associated with selective forces effecting survivalrate. In this connection, Fujio *et al.* (1) reported that the released population of the plaice showed an excess of heterozygotes at *Idh-1* locus and genotypes were associated with marked differences in growth rate during the culture period. Therefore, systematic analysis of heterotic advantage is needed in fishery management.

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